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Ecosystem metabolism of benthic and pelagic zones of a shallow productive estuary: Spatio-temporal variability

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Running head: Estuarine habitat metabolism

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Abstract

Long-term deterioration of water quality is known to reduce the relative importance of benthic to pelagic ecosystem metabolism in shallow coastal ecosystems, but drivers of spatial and short-term variability in ecosystem metabolism are poorly understood. We address this knowledge gap through detailed seasonal measurements of ecosystem metabolism across depth gradients from shallow (2–3 m), eelgrass-dominated to deeper (4–5 m), muddy regions of a shallow, productive estuary. Combined measurements of gross primary production (GPP), respiration (R) and, by difference, net ecosystem production (NEP) by the open water diel oxygen technique and *in-situ* chamber incubations showed high importance of shallow eelgrass habitats for metabolism at the system scale. Seasonal variations in GPP, R and NEP increased with light availability and temperature with highest NEP in all habitats during the warm and sunny mid-summer. The shallow eelgrass-dominated and neighboring habitats were seasonally net autotrophic (NEP = 0.54 and 0.31 mg O_2 m^{-2} d^{-1}), compared to net heterotrophy (NEP = -0.26 mg O_2 m^{-2} d^{-1}) at the deeper, muddy site. Detailed studies along depth gradients further confirmed the role of eelgrass as a key driver of spatial differences in ecosystem metabolism across the estuary. Strong northerly winds (>8 m s^{-1}) caused short-term (< 24 h) periods of similar oxygen dynamics and similar apparent productivity in shallow and deeper waters, indicative of efficient lateral mixing while calm periods (< 4 m s^{-1}) enabled formation of "pockets", i.e. water masses with limited connectivity, which exacerbated the metabolic differences between shallow and deep sites.

Introduction

Benthic primary producers such as seagrasses play important ecological roles as hotspots for production, storage and export of organic carbon (Duarte et al. 2013, Duarte 2017) in addition to

efficiently retaining nutrients, stabilizing sediments and stimulating biodiversity in shallow coastal ecosystems (e.g. Hemminga & Duarte 2000). The relative importance of benthic and pelagic primary producers in shallow estuaries is largely a function of water clarity, and strongly related to nutrient loading (Borum & Sand-Jensen 1996). Shallow coastal seafloors typically have a high cover of benthic macrophytes in the well-lit photic zone, where they can contribute significantly to gross primary production (Duarte & Chiscano 1999, McGlathery et al. 2001, Öberg 2006). Reductions in water clarity of shallow coastal waters, mostly due to eutrophication, have caused global losses and reduced depth colonization of seagrass meadows (Short & Wyllie-Echeverria 1996, Orth et al. 2006, Waycott et al. 2009). Historically, most of the Danish estuaries were dominated by the seagrass *Zostera marina* (eelgrass), but following the wasting disease in the 1930s, and partial recovery thereafter, the extent and depth distribution of eelgrass decreased markedly, as eutrophication reduced water clarity (Nielsen et al. 1992, Boström et al. 2014). For example, Limfjorden in Denmark experienced a shift from a pristine, benthic macrophyte-dominated clear water regime with high total gross primary production (GPP) in the early twentieth century to an eutrophic, plankton-dominated regime still with high total GPP in the 1980s when nutrient loadings peaked (Krause-Jensen et al. 2012). Nutrient loadings and concentrations in Danish coastal waters have decreased significantly since the 1980s, but improvements in water clarity and eelgrass depth colonization have been slow with only recent signs of recovery process starting (Riemann et al. 2016). While the eutrophication-induced transition from a benthic to a pelagic dominated system is well documented, the reversal – oligotrophication is less well understood (Duarte et al. 2009, Riemann et al. 2016), partly because of multiple feedback mechanisms, including unsuitable soft and organic-rich substratum, that potentially delay the return of the vegetation (Maxwell et al. 2017). A few recent studies,

however, present trajectories of recovery, and changes in overall productivity with a reversal to a benthic dominated system (McGlathery et al. 2012, Riemann et al. 2016, Staehr et al. 2017).

In shallow coastal ecosystems such as Roskilde Fjord, Denmark, where sufficient light reaches the sediment surface to support benthic primary production in most of the estuary (Staehr & Borum 2011), net ecosystem production (NEP) is expected to be autotrophic (NEP > 0) with GPP exceeding ecosystem respiration (R) (Ziegler & Benner 1998, Kemp & Testa 2011). This expectation agrees with observed long-term improvements in water clarity and greater depth limits of the dominant macrophyte, *Zostera marina*, associated with increasingly autotrophic conditions (Staehr et al. 2017). Although several studies have assessed the relative roles of pelagic and benthic compartments in shallow aquatic ecosystems (e.g. Borum & Sand-Jensen 1996, Krause-Jensen et al. 2012, Van de Bogert et al. 2007), only few studies have allowed a direct partitioning of the metabolic processes (e.g. Caffrey et al. 1998, Hume et al. 2011, Murrell et al. 2017). Moreover, the relative contribution of benthic versus pelagic primary producers along depth gradients and the temporal dynamics in ecosystem metabolism are poorly understood (Kamp-Nielsen 1992, Kemp & Testa 2011).

Moving from shallow well-illuminated to deeper shaded habitats will likely shift net ecosystem production from autotrophy to heterotrophy with increasing plankton respiration and decreasing benthic photosynthesis. In support of this, daily variation of oxygen and temperature show declines with depth, indicative of higher metabolic rates in the near-shore shallower waters related to the presence of rooted autotrophs (Odum 1967, Swaney 1999). The partitioning of total ecosystem respiration between planktonic and benthic components is also expected to change with depth, as planktonic processes are strongly favored in deeper systems (e.g., Kemp et al.1992, Heip et al. 1995). Moreover, temporal variation in external forcing variables, including

89 sunlight, wind, meteorological tides, and stream flow may cause significant day-to-day, and even
90 hourly variability in primary production, respiration, and net ecosystem production (Jennings et
91 al. 2012, Staehr & Sand-Jensen 2007). Short-term hourly increases in wind-induced water
92 turbulence can, hence, lead to higher rates of sediment resuspension, reducing water clarity, light
93 penetration, and photosynthesis (Arfi et al. 1993) as well as reintroduction of labile organic
94 matter into the water column, stimulating planktonic respiration (Demers et al. 1987). Large
95 daily variability in ecosystem production has been associated with variations in cloud cover and
96 the resulting changes in sunlight (e.g., Fisher et al. 2003), as well as with wind-induced changes
97 in mixing and resuspension (Staehr & Sand-Jensen 2007). Depending on water depth, sediment
98 characteristics and density of macrophytes, such external forcing will interact with the complex
99 local hydrodynamics associated with the canopy structure, eventually influencing the oxygen
100 dynamics and metabolic processes in nearshore shallow habitats differently (Hume et al. 2011).

101 In this study, we utilized high-frequency open-water measurements of oxygen, temperature,
102 salinity and wind speed to investigate temporal and spatial variability in estuarine metabolism at
103 sites with different benthic habitats and at different water depth. This enabled us to characterize
104 the seasonality in GPP, R and NEP and investigate the regulatory importance of different
105 external conditions over different time scales. A series of benthic and pelagic oxygen *in-situ*
106 incubation studies furthermore allowed assessment of the benthic and pelagic contributions to the
107 ecosystem-integrated rates. Multiple measurements along a depth gradient made it possible to
108 test expectations of larger magnitude of variability of ecosystem metabolism in shallow eelgrass-
109 dominated areas compared to deeper muddy parts of the estuary. These measurements along a
110 depth gradient ultimately enabled us to evaluate the influence of meteorological forcing events
111 for short-term oxygen dynamics in the coastal environment.

Methods and materials

Study sites

Our study was conducted in the southern, inner part of Roskilde Fjord, a 30 km long, shallow estuary (mean depth 3 m, surface area 123 km^2) in north Zealand, Denmark (Fig. 1). The outer part connects to the Kattegat through the Isefjord, while a sill restricts water transport between the outer and inner part of the estuary. The inner part is slightly shallower than the outer but has a larger surface area, which overall results in 23% larger volume. The water column in the inner part of the estuary is well-mixed, with only short sub-diel stratification. Depending on the strength of wind driven exchanges, the average water residence time in the inner part of the estuary ranges between 90 and 720 days (Kamp-Nielsen 1992, Josefson & Rasmussen 2000). Land use in the catchment is dominated by agriculture (67%) whereas urban areas account for 15% and the remaining watershed is covered with forests, wetlands, and lakes. Roskilde, the largest city in the catchment, lies on the shore of the inner basin. Historically, Roskilde Fjord, like the rest of Denmark, had very extended eelgrass meadows (Petersen 1901, Steemann-Nielsen 1951), and while these were severely reduced by eutrophication, peaking in the 1980s, Roskilde Fjord and Denmark are still hotspots of eelgrass distribution and experiencing recent increases in eelgrass depth distribution (Riemann et al. 2016, Staehr et al. 2017).

Temporal variability in ecosystem metabolism was investigated through continued open system diel O_2 measurements as well as through episodic benthic and pelagic incubations in three main sites in the inner part of Roskilde Fjord from April (spring) to December (winter) 2015 (Fig. 1, Table 1). Two of the sites were located at 2-3 m depth, one representing an eelgrass meadow ("Eelgrass") and another, approximately 100 m to the south, representing a neighboring non-

eelgrass site. The latter is referred to as the "Bare" site, although the site had scattered small stones (5 – 10 cm in diameter) covered by filamentous algae. The third site ("Muddy") represented the deeper (5 m) area characterized by fluffy organic-rich sediment with no benthic vegetation. In our study, we distinguish between eelgrass dominated vs. bare vs. mud dominated areas and refer to these as different habitats due to their marked differences in substrate and vegetation. Furthermore, to quantify short-term effects of physical forcing, we conducted a gradient study in May 2016 along a transect perpendicular to the shoreline (from ~100 to 500 m off the shore) starting at 1.8 m and reaching ~5 m depth and representing all three habitat types (Table 1, Fig. 1).

Water quality sampling

Water quality sampling for measurements of total and dissolved inorganic nutrients, phytoplankton biomass (chlorophyll a; Chl *a*), Secchi depth and profiles of temperature, salinity and oxygen was carried out every two weeks from May to December 2015 at the central muddy station, which coincided with a monitoring site (Fig. 1; St. 60) within the Danish National Aquatic Monitoring and Assessment Program (DNAMAP). Sampling and measurements of dissolved inorganic nitrogen (DIN; $NO_3^- + NO_2^- + NH_4^+$), dissolved inorganic phosphorus (DIP; PO_4^{3-}) and Chl *a*, followed standard technical guidelines (Kaas & Markager 1998). Water column concentrations of nutrients were measured at 1 m depth by colorimetric methods (Danish Standards Association, 1975, 1985). Chl *a* was analyzed from Whatman GF/F or GF 75 Advantec filtered samples from 1 m depths and extracted in ethanol according to Strickland and Parsons (1972). To supplement these monitoring data, water samples were taken from 1 m depth on a biweekly to monthly basis to measure pelagic primary production using a modified oxygen incubation technique as described below.

158 *Field measurements of metabolic rates and physical forcing*

159 Diel oxygen measurements: Rates GPPR and NEP were determined from changes in the
160 concentration of dissolved oxygen (DO) following the diel oxygen technique as originally
161 described by Odum (1956). In our study, continuous measurements (every 10 min) of DO and
162 water temperature were made with miniDOT oxygen optode loggers (Precision Measurement
163 Engineering, Vista, CA, USA, in the following termed "oxygen logger") at the three main sites.
164 Photosynthetically active radiation (PAR) was measured simultaneously at the same frequency
165 using four Odyssey PAR loggers (Dataflow Systems Pty Limited, henceforth termed "PAR
166 loggers") placed within a 2 meter depth range at all three sites. Odyssey loggers were calibrated
167 based on parallel measurements of PAR by a LICOR® 2π sensor. All sensors were cleaned on a
168 bi-weekly basis to reduce sensor drift from fouling. Fouling of sensors was further reduced by a
169 copper mesh for the oxygen sensors and by a copper film placed around the light sensors. Sensor
170 drift was corrected by assuming a linear decline in sensitivity over each bi-weekly period.

171 From April to December 2015, we applied two oxygen loggers and a PAR logger at each site
172 from April to December 2015 (Table 1). At the deeper muddy site, oxygen loggers were attached
173 to a surface buoy approximately one meter below the sea surface while at the shallow sites
174 sensors were fixed to metal poles secured firmly into the sediment, measuring approximately 30
175 cm above the seafloor. We do not anticipate any effects of sonde placement above sediment as
176 the water column was fully mixed at all three sites throughout the measurement period. At each
177 site we calculated the diffuse light attenuation coefficient (K_{PAR}) as the linear slope between
178 depth and the log of light intensity. Regressions with a poorer fit ($r^2 < 0.8$) were excluded from
179 further analysis.

180 The supplementary short-term study of diel O_2 variability represented a four-week period from
181 26th of April to 20th of May 2016 (Table 1), we deployed five oxygen loggers, and one YSI
182 6600V2® multisonde measuring oxygen, temperature, water level and salinity. The equipment
183 was placed along a depth gradient from 1.8 m inside the eelgrass meadow extending
184 approximately 500 m perpendicular to the shoreline, northwest towards the muddy site at 5 m
185 depth. All sensors were attached to metal poles and positioned ~30 cm above the sediment
186 surface.

187 Benthic incubations: To investigate the importance of eelgrass meadows and other benthic
188 components for diel variations in oxygen concentrations and daily water column net fluxes, we
189 used *in-situ* chambers, which allowed us to incubate a section of the seafloor with its benthic
190 community. Triplicate chamber incubations were done at the three types of habitat in April
191 (spring), June (early summer) and August (mid/late summer) 2015, corresponding to mean
192 temperatures of 9, 16 and 21°C, respectively. Chambers consisted of gas impermeable
193 transparent plastic bags (19 x 42 cm; diameter x height = 12 L) attached to a hard PVC collar,
194 which was secured firmly into the sediment with metal plugs (Fig. 2A). Incubations were made
195 over a period of approximately 24 hours with logging of oxygen, temperature and light every 10
196 minutes by an oxygen logger and a light logger placed at a depth of about 30 cm above the
197 seafloor inside each chamber fixed to a metal stick placed into the sediment.

198 Pelagic incubations: Water samples were collected on 14 occasions from April to December at 1-
199 2 m depth at the deeper muddy site. These pelagic rates were assumed to also represent pelagic
200 rates at the shallower bare and eelgrass sites. Triplicate measurements of pelagic metabolism
201 were done by *in-situ* incubation of the sampled water in 0.5 L transparent glass jars attached to a
202 buoy over approximate 24 hours at 1 m depth. An oxygen logger was inserted 5 cm into each

glass jar and recorded oxygen and temperature every 10 minutes. Next to these jars, a PAR logger recorded light simultaneously within the same frequency.

Modelling of oxygen fluxes

To improve comparisons of metabolic rates between open-water and chamber measurements, we applied a similar inverse modeling approach (Hanson et al. 2008, Brigenthi et al. 2015), which utilizes data on irradiance and temperature to model metabolic rates from high-frequency oxygen measurements. This approach has less assumptions regarding constant respiration and facilitated more realistic daily rates using information on the ambient light and temperature conditions during similar periods. This approach minimizes possible mismatch between open-water and chamber fluxes that may result from differences in light and temperature conditions during sampling.

Diel changes in DO for open-water measurements, pelagic bottle incubations and benthic chambers were modelled using equation 1:

$$DO_{[t+1]} = DO_{[t]} + GPP_{[t]} - R_{[t]} + F_{[t]} \quad (1)$$

where $DO_{[t+1]}$ and $DO_{[t]}$ are the DO concentrations (mg L^{-1}) at discrete times t + 1 and t with 10 min resolution; $GPP_{[t]}$ is the gross primary production at time t; $R_{[t]}$ is the ecosystem respiration at time t (eq. 2); and $F_{[t]}$ is the net exchange of O_2 between the lake and the atmosphere at time t (eq. 3). For bottle and chamber incubations with no air-water gas exchange, eq.1 was simplified by removing the $F_{[t]}$ term. Net ecosystem production (NEP) was calculated using a light and temperature dependent model (eq. 2) described by Brighenti et al. (2015):

$$\Delta DO_{t+1} = NEP_{hr} = P_{max} \times tanh(\alpha \times I_t / P_{max}) - R_{20} \times 1.07^{T_t - 20} \quad (2)$$

where P_{max} is the light saturation point, α is the initial linear slope of the photosynthesis vs. light relationship describing the average rate of photosynthesis per unit of PAR, I_t is the surface PAR (µmol photons m^{-2} s^{-1}) measured at time t. Respiration was a function of the rate of R at 20℃, water temperature (T_t) and a temperature sensitivity constant equal to 1.07.

Net oxygen exchange between air and water was calculated according to Staehr et al. (2010):

$$F_{[t]} = (k_{[t]} (DO_{[t]} - DO_{sat[t]})/Z)/2 \quad (3)$$

where DO is the measured concentration in the water and DO_{sat} is the concentration in water at equilibrium with the atmosphere at ambient temperature and pressure (Weiss 1970) at time t, $k_{[t]}$ is the coefficient of gas exchange for DO at time t and Z is the total depth of the water column at the measurement site. k was calculated based on the relationship among Schmidt numbers (Sc) and temperature (Jähne et al. 1987): $k = k_{600} \times (Sc/600)^{-0.5}$, where k_{600} is the piston velocity calculated as function of wind speed at 10 m height according to a model developed for Roskilde Fjord (Mørk et al. 2014). Wind speeds were measured at a meteorology mast 1 km away from the experimental area.

The three parameters in equation 2 (P_{max}, α and R_{max}) were estimated using a conjugate-gradient optimization algorithm that minimized the sum of squared errors between estimated and observed DO for each incubation period with constraints on the parameter space, i.e. lower and upper bound for each parameter. The parameters P_{max}, α, and R_{max} were finally used to estimate DO concentrations at every 10 minutes using equation 1 above. Parameterization of DO curves can be poor on days where oxygen dynamics are dominated by physical exchanges (Hanson et al. 2008). To assess model performance (i.e., how well the model fitted the observed DO data), we determined the coefficient of determination (r^2). To reduce bias by erroneously modeled DO

curves, we removed sonde days with r^2 below 0.2 (Obrador et al. 2014) resulting in removal of 15% of the 1068 sonde days measured in total.

The optimized parameters, P_{max}, α and R_{max}, were subsequently used to calculate hourly rates of NEP, GPP and R inserting 10 min interval recordings of the mean available light in the water column and water temperature over a 24 h period. Mean light availability (E_{mean}) for primary producers (*i.e.* the expected value of PAR to which an algal cell is exposed, assuming that the water column is fully mixed) was calculated according to Staehr & Sand-Jensen (2007):

$$E_{mean} = E_0(1 - e^{-K_{PAR}Z})/(K_{PAR}Z) \quad (4)$$

where K_{PAR} is the diffuse PAR attenuation coefficient (m^{-1}), Z is the total depth of the water column, and E_0 is the surface PAR (µmol photon m^{-2} s^{-1}) measured at the nearby (1 km) meteorological mast.

Daily rates of GPP, NEP, and R were calculated as the average hourly rates (over 24 hours) multiplied by 24 hours. Volumetric daily rates were then converted to area-specific rates. For open-water measurements and bottle incubations, this simply involved multiplying volumetric rates (g O_2 m^{-3} d^{-1}) with the depth (m) of the water column at the measurement site. For benthic chamber incubations, this conversion involved multiplying with the volume (12 L on average) of the incubation bags and dividing by the area (0.028 m^2) of the incubation chambers. We display respiration rates as negative values to facilitate rate comparison.

Statistical analysis

To enable a comparison of open-water (total), pelagic and benthic estimates of GPP, R and NEP, we applied a general linear mixed model (GLMM) describing spatial variation among habitats and seasonal (at a monthly resolution) variation as fixed factors, and variation among sampling days within months as random factors in addition to residual variation. Since data on metabolic rates were not consistently measured throughout the entire study period for all three habitats (muddy, bare and eelgrass), marginal means were calculated from the GLMM to produce comparable means for spatial and seasonal variation. The significance of the fixed effects in the GLMM was tested using an F-test for the additional variation explained by the given factor, i.e. comparing the models with and without the factor. Test for differences in metabolic rates among habitats were performed as contrasts between parameter estimates. The GLMM was analyzed using PROC MIXED in SAS v. 9.3. Furthermore, the short-term temporal variability in GPP, R and NEP for the open-water estimates was estimated by calculating the standard deviation of the residuals between observations and a 15-day moving average across the entire study period. Statistical investigation of relationships between variables was performed using a simple Spearman ranks correlation analysis on theuntransformed data. Finally, to evaluate the level of background respiration (*sensu* Solomon et al. 2013) associated with particulate and dissolved organic matter, we performed a linear regression model of R as a function of GPP (using geometric means), and estimated the background respiration level as the y-intercept.

Results

Seasonal changes in physical and chemical conditions

Depth profiles showed that the water column was fully mixed from April to December with similar temperature and salinity (data not shown) at surface and bottom at the deeper muddy station (Figure 3A). Daily surface irradiance showed large day-to-day variation and peaked around the equinox in late June, approximately two months before the peak of water temperatures (Figure 3A). Daily wind speeds averaged 4.4 m s^{-1} (range: 1.2 - 9.7 m s^{-1}), with 47% of days having wind speeds <4 m s^{-1} and only 2 % having wind speeds >8 m s^{-1} (Figure 3B). Chl *a* levels were overall low (range: 0.9 to 8.9 µg L^{-1}) and Secchi depths relatively high (range: 3.2 to 5.1 m ~ visible at bottom), considering the rather high nutrient levels (Figure 3C). Secchi depth was strongly related to Chl *a* (r_s = -0.54, p = 0.008). Chl *a* peaked in late April indicating a late spring bloom (Figure 3C) with a corresponding drawdown of DIN. Nitrogen remained almost completely depleted until late August when a small increase occurred (Figure 3D). Following a phytoplankton bloom in September (Figure 3C), DIN levels declined. During the DIN-depleted summer period, DIP increased, followed by decreasing concentrations in late autumn as DIN accumulated (Figure 3D).

Temporal pattern of ecosystem metabolism in different habitats

All three habitats exhibited a clear seasonality in open-water estimates of GPP, NEP, and R (Fig. 4; Table 2). Rates of primary production and respiration increased during spring, reaching maximum in late summer and declining in fall and winter (Fig. 4). GPP increased more strongly with water temperatures and solar radiation than R (Table 3). While GPP seemed unrelated to wind at the deepest site, there was a significant correlation between respiration rates and wind speeds for all three habitats. In combination, these relationships indicate that net autotrophy (NEP > 0)

312 prevailed during warm, sunny and calm periods (Table 3). Rates of ecosystem respiration were
313 weakly correlated with GPP in the two shallow habitats (r_s = 0.34, $p < 0.001$) compared to a
314 stronger correlation in the muddy habitat (r_s = 0.63, $p < 0.001$). Regression analysis of open water
315 R vs GPP showed that the background respiration level was very low as it was not significantly
316 different from zero (Students t-test, $p > 0.05$) in any of the habitats.
317
318 There were overall significant differences in open water metabolic rates among the three habitats
319 (Table 2). Areal GPP was significantly lower in the deeper muddy habitat (4.60 ± 0.59 g O_2 m^{-2} d^{-1}
320 ; mean ± 95%CL) compared to the eelgrass (5.26 ± 0.23 g O_2 m^{-2} d^{-1}) and bare habitats (5.40 ±
321 0.23 g O_2 m^{-2} d^{-1}). Area respiration rates were slightly higher in the bare habitat (5.13 ± 0.33 g O_2
322 m^{-2} d^{-1}) compared to the eelgrass (4.71 ± 0.32 g O_2 m^{-2} d^{-1}) and muddy habitats (4.84 ± 0.66 g O_2
323 m^{-2} d^{-1}). The metabolic balance (NEP = GPP – R) was overall positive for the eelgrass (0.54 ± 0.36
324 g O_2 m^{-2} d^{-1}) and bare habitats (0.31 ± 0.36 g O_2 m^{-2} d^{-1}) but negative for the muddy habitat (-0.26
325 ± 0.64 g O_2 m^{-2} d^{-1}) supporting expectations of net autotrophy in the vegetated area and net
326 heterotrophy in the non-vegetated deeper muddy area.

327 Days of net heterotrophy (NEP < 0) occurred during all months for all three habitats (Fig. 4), but
328 were slightly more frequent at the muddy site (48%) compared to the shallower sites (44%). While
329 negative NEP occurred in all months, the shallow sites mostly experienced net heterotrophy during
330 autumn, suggesting a significant loss of autotrophic biomass here. The analysis of residuals from
331 the 15-day moving average trend showed higher day-to-day variability in open-water metabolic
332 rates in the muddy habitat, especially for GPP and R (cf. Fig. 4). Standard deviations of the
333 residuals from the muddy habitat were 2.71, 2.67 and 2.74 g O_2 m^{-2} d^{-1} for GPP, R and NEP. In
334 comparison, standard deviations of the residuals in the bare habitat were 1.63, 2.43 and 2.63 g O_2

335 m^{-2} d^{-1}, almost similar to those of the eelgrass habitat (1.65, 2.30 and 2.50 g O_2 m^{-2} d^{-1} for GPP, R
336 and NEP, respectively).

337 *Contribution of pelagic and benthic habitats to ecosystem integrated rates*
338 Based on the bottle incubations, we determined areal-specific rates of pelagic GPP, R and NEP,
339 which were much lower than rates obtained with the open-water measurements and the benthic
340 chamber incubations (Fig. 5). Pelagic primary production for the entire period (April to
341 December) was on average 2.0 g O_2 m^{-2} d^{-1}, compared to pelagic respiration rates of 1.6 g O_2 m^{-2}
342 d^{-1}, indicating that the water column was overall net autotrophic (0.4 g O_2 m^{-2} d^{-1}). Pelagic GPP
343 estimates suggested moderate seasonal variation in productivity (Fig. 6), although the statistical
344 test showed no significant difference between months (Table 2).

345 Benthic production and respiration rates, measured in benthic incubation chambers, were overall
346 much higher in the eelgrass beds compared to the nearby shallow bare sediment and the deeper
347 muddy site (Table 4). The eelgrass site was the only habitat with a net positive flux of oxygen
348 (NEP > 0) from the sediment to the water column (Table 4). Low metabolic rates in the eelgrass
349 habitat in April were measured during strong winds (> 8 m s^{-1}), low irradiance and highly turbid
350 cold water, possibly explaining the much lower rates compared to other months (Fig. 6). Benthic
351 rates of both GPP, R, and NEP all showed significant seasonal variation with significant
352 differences among the three sites (Table 3). For GPP and NEP, there was significant interaction
353 between habitats and seasons, indicating that seasonal variation differed among habitats.

354 Comparing open-water (~total) seasonal average estimates of GPP, R and NEP with estimates
355 based on *in-situ* benthic chambers (~benthic) and bottle incubations (~pelagic), showed a close
356 (~100%) agreement between total and the sum of benthic + pelagic rates for the shallow eelgrass

site (Fig. 5). Interestingly, although the bare shallow site had total metabolic rates very similar to the nearby eelgrass site, the sum of the measured benthic and pelagic rates could only account for 37% of the open-water GPP estimates and 54% of the ecosystem respiration rates measured at the bare site (Fig. 5). A similar discrepancy was found for the muddy site where the sum of benthic and pelagic metabolic rates only accounted for 46 and 47% of open-water GPP and R, respectively. This discrepancy between open-water and chamber-based rates for the bare and muddy sites seemed connected to the substantially lower benthic rates measured in these habitats, compared to the eelgrass-dominated habitat where benthic rates were multifold higher (Fig. 5). However, integrating across all months and habitats with the GLMM, total rates of GPP, R and NEP were only approximately 10% higher than the sum of pelagic and benthic rates, suggesting a relative good agreement between estimated rates. While pelagic and benthic processes showed different seasonality, their overall contribution to total rates were similar with 54, 56 and 46% of GPP, R and NEP, respectively, accounted for by benthic processes.

Changes in metabolic rates with depth and wind conditions

Deploying an array of oxygen, light and temperature loggers for four weeks from the near-shore shallow (1.8 m) to the deeper central site (5 m), enabled us to calculate daily rates of GPP, R and NEP along a depth gradient (Fig. 7). Although volumetric rates were three-fold higher at the shallow sites, water column-integrated rates were more similar along the gradient. Nevertheless, shallow sites had almost twice as high areal GPP and R rates, with small albeit positive and significant NEP compared to NEP ~ 0 at the deeper sites.

As two of our oxygen loggers in the 3 - 4 m depth interval malfunctioned, our characterization of the depth gradient was not as detailed as planned. Aggregating data into a near-shore (1.8 to 2.1 m depth) and deep (4.1 to 5 m depth) area, allowed us to investigate the importance of short-term

wind and water movement events for diel changes in oxygen and the derived daily rates of ecosystem metabolism at contrasting depths (Fig. 8). During the four-week measurement campaign, considerable variation in wind speed, wind direction, water level and surface light was observed. The relatively long, narrow and shallow hydromorphology of the estuary, oriented in a south-north direction (Fig. 1), makes water level in the inner part sensitive to northerly winds, forcing water from the outer broad into the shallow inner broad, where our study was carried out. This wind-driven increase in water level was clearly observed on three occasions during our measurement campaign, and the increases in water levels scaled with the duration and strength of northerly winds (Fig. 8AB). In the first event, relatively weak northerly winds (4 to 5 m s^{-1}) only persisted for two days causing a water level rise of 0.3 m (Fig. 8AB). The second event lasted for three days with similarly weak winds, causing a water level rise of 0.4 m. The last event lasted five days but with winds ranging between 6 to 12 m s^{-1}, resulting in a water level rise of 0.5 m lasting for three days (Fig. 8AB). During this last event, observed *in-situ* light levels dropped considerably. Although these meteorological events were short-lived, they had marked effects on the variability and magnitude of DO at the shallow sites (Fig. 8C). Large daily variability in oxygen ranging from 80 to 200 % saturation were immediately reduced to variations similar to the deeper offshore sites with diel oxygen variations ranging between 80 and 110 % saturation (Fig. 8C). Consequently, daily rates of GPP were lower on days with reduced oxygen dynamics, with the duration and strength of the wind-driven water movements driving rates down (Fig. 8D). This indirect effect of wind on apparent ecosystem productivity was also evident when plotting GPP and NEP against daily mean levels of wind speed (Fig. 9). While productivity at the deeper sites appeared to be unaffected by wind conditions, winds had a significant negative effect on both apparent GPP and NEP at the shallow sites becoming net heterotrophic at winds above 8 m s^{-1}.

Discussion

This study provides a direct comparison of integrated ecosystem metabolism rates in three functionally different habitats within a temperate shallow estuary. Seasonal application of the diel oxygen technique in combination with incubations of benthic versus pelagic compartments at different depths furthermore enabled us to evaluate conditions affecting temporal variability along a depth gradient, and to quantify the relative importance of benthic and pelagic compartments.

Temporal variability in open-water metabolic rates

Our modelled ecosystem metabolism from continuous oxygen measurements showed large variations in GPP, R and NEP on a daily basis and across seasons and habitats. The magnitude and temporal variability in open-water metabolic rates resembled those found for North American estuaries (Caffrey 2004). Similar to Caffrey (2004), we found that the balance between GPP and R was largely determined by the habitat type. Accordingly, our shallow sites inside or just outside the eelgrass meadows were significantly more net autotrophic as compared to the more net heterotrophic, deeper muddy site with greater dominance of pelagic processes. As observed in other studies in shallow estuaries and lakes, our daily NEP estimates indicated large shifts from net autotrophy to net heterotrophy over both seasonal (monthly) and even daily time scales (eg. Staehr & Sand-Jensen 2007, Kemp & Testa 2011). At the central deeper station, there was a general pattern of spring net autotrophy transitioning to summer heterotrophy, consistent with a long-term analysis of net oxygen exchanges in Roskilde Fjord (Staehr et al. 2017). In comparison, the shallow sites showed less seasonality in NEP remaining mostly positive throughout the year, although with a clear drop in productivity during late autumn when light and temperature decreased and storm-related loss of vegetation occurred. Our residual analysis showed that day-to-day variability was clearly larger at the central pelagic-dominated station for both GPP and R

426 and to a lesser extent for NEP. This suggests that deeper stations dominated by pelagic processes
427 and with stronger light limitation are more sensitive to changes in nutrient conditions and incoming
428 light.
429
430 *Ecosystem integrated metabolic rates*
431 Across the three investigated habitats, summer (June through August) mean daily rates of GPP in
432 Roskilde Fjord (7.0 ± 0.3 mg O_2 m^{-2} d^{-1}; Mean ± SE) are slightly lower than those reported by
433 Hoellein et al. (2013) for a large range of estuaries across the Northern hemisphere (~10 ± 1 g O_2
434 m^{-2} d^{-1}). Interestingly respiration rates in Roskilde Fjord are less than half (6.0 ± 0.3 g O_2 m^{-2} d^{-1})
435 than those reported by Hoellein et al. (2013): (~14 ± 1 g O_2 m^{-2} d^{-1}). Thus, where many estuaries
436 display strong net heterotrophy, even during summer, Roskilde Fjord is overall net autotrophic
437 (1.0 ± 0.3 g O_2 m^{-2} d^{-1}) in agreement with a small subset of shallow densely vegetated estuaries
438 around the USA (Caffrey 2004) and Chesapeake Bay tributaries (Kemp & Testa 2011). Our results
439 are similar to those of Hume et al. (2011) who applied the eddy correlation technique to measure
440 productivity during summer (June and July), reporting NEP in vegetated shallow areas of 0.6 g O_2
441 m^{-2} d^{-1}.
442
443 Using a molar O_2 : C quotient of 1, and using a simple polynomial model (Laas et al. 2012) to
444 extend the measured GPP period (April to December) to cover the entire year, Roskilde Fjord had
445 an annual GPP ranging between 575 and 663 g C m^{-2} y^{-1} across the muddy and eelgrass habitats.
446 These values are in the high end of the spectrum compared to GPP values reported by Borum &
447 Sand-Jensen (1996) for 34 coastal systems (median 346; range 28 to 820 g C m^{-2} y^{-1}) and the
448 nearby Limfjorden where GPP was estimated to have reached 350 g C m^{-2} y^{-1} during pristine

449 conditions (Krause-Jensen et al. 2012). Annual NEP ranged from -35 g C m^{-2} y^{-1} in the muddy
450 area to 74 g C m^{-2} y^{-1} in the vegetated area, similar to previous findings by Caffrey (2004) using
451 the open-water oxygen technique (range -55 to 68 g C m^{-2} y^{-1}) in five shallow estuaries dominated
452 by submerged aquatic vegetation. In comparison, net primary production, estimated from net
453 accumulation of plant biomass, may be several times higher within dense beds of perennial
454 macrophytes (Borum & Sand-Jensen 1996). For example, Wium-Andersen & Borum (1984)
455 estimated annual (April to October) net above-ground production of 814 g C m^{-2} y^{-1} in a Danish
456 estuary. The significantly lower NEP rates obtained from the open-water oxygen measurements
457 are not surprising as this technique accounts for oxygen production and consumption by both auto-
458 and heterotrophs occupying both benthic and pelagic components (Staehr et al. 2012b). In addition,
459 depending on water exchange, the oxygen technique will integrate processes that can easily exceed
460 the area of the densely vegetated macrophyte beds (Hume et al. 2011). In support of this, NEP
461 rates measured within benthic chambers under calm wind conditions (2 to 4 m s^{-1}) in June were up
462 to 4 times higher than NEP rates calculated from parallel open-water oxygen measurements within
463 the dense eelgrass bed.
464
465 *Importance of benthic and pelagic contributions*
466 To distinguish benthic and pelagic contributions to total areal rates of GPP, R and NEP, we applied
467 24-hour oxygen chamber incubations and compared these with daily rates calculated from open-
468 water oxygen measurements. The analytical approach was essentially the same for benthic, pelagic
469 and open-water measurements, with the modification that incubations did not account for air-water
470 gas exchange.
471

Pelagic rates of metabolism measured in this study were similar to rates reported by Mantikci (2014) for the central part of Roskilde Fjord, where a combination of ^{14}C and oxygen incubation techniques was used. Converting rates by Mantikci (2014) using a molar O_2 : C quotient of 1, GPP ranged between 0.3 and 5.3 mg O_2 m^{-2} d^{-1}; R between 0.3 and 3.2 mg O_2 m^{-2} d^{-1} and NPP between 0.1 and 3.5 mg O_2 m^{-2} d^{-1}. These levels are consistent with those reported here (Figure 6) and both studies report similar seasonality with peaks in June and July. Oxygen fluxes across the water-sediment interface were measured in light and dark incubations of sediment cores as part of the DNAMAP, including St. 60 in Roskilde Fjord (Dalsgaard 2003). Dalsgaard (2003) reported seasonal ranges in GPP from 0.1 to 1.5 g O_2 m^{-2} d^{-1}; respiration from 0.3 to 2.0 g O_2 m^{-2} d^{-1}, and NEP from -0.7 to 0.5 g O_2 m^{-2} d^{-1}. These levels resemble those measured for the muddy station (Table 4) with a monthly variation mirroring that of water temperature (Figure 6). Thus, pelagic and benthic rates reported in this study have similar levels and temporal variability as those previously reported.

Comparing open-water (total) rates with pelagic and benthic incubations, demonstrated that benthic metabolism is important and often dominating in shallow coastal environments. This was particularly prominent in the eelgrass area where the benthic habitat alone accounted for approximately 90% of total GPP and R and 64% of NEP. While the unvegetated shallow site had total metabolic rates very similar to the nearby eelgrass site, the sum of the measured benthic and pelagic rates could only account for 37% of the open-water GPP estimates and 54% of the ecosystem respiration rates (Fig. 5). An even greater discrepancy between total and the sum of pelagic and benthic rates was found for the muddy site. We attribute this difference to the metabolic footprint of the eelgrass meadow markedly exceeding beyond the confines of the

meadow, thereby supplying oxygen to the neighboring unvegetated site as well as the deeper central sites. We interpret the observed systematic differences in metabolism between areas differing substantially in substrate conditions and vegetation cover, mainly as differences between different habitats, rather than just spatial differences related to factors such as differences in water depth. This terminology agrees with Caffrey (2004) who also categorized her sites based on the dominant habitat adjacent to the deployment site.

The importance of benthic processes was not surprising given the shallowness of the estuary and its relatively clear waters, providing sufficient light at the sediment surface supporting benthic primary production throughout the studied areas. Previous evaluations of the benthic vs. pelagic contribution to the inner part of Roskilde Fjord suggested that phytoplankton contributed between 100 and 62% of total areal GPP (Borum & Sand-Jensen 1996). However, water quality has improved significantly since the 1990s, especially in the inner parts of the estuary (Staehr et al. 2017). Accordingly, Chl *a* has decreased by 50% and Secchi depth has increased by approximately 1 m enabling eelgrass to expand from 2 to 2.6 m depth, and the improved light environment has likely stimulated the growth of benthic algal communities (Sand-Jensen & Borum 1991, Staehr et al. 2017). It is therefore plausible that the benthic compartment now plays a much larger role compared to the 1990s. This change should render the system more autotrophic and a greater sink for carbon and nutrients (McGlathery et al. 2007, Fourqurean et al. 2012) stored in the sediments by accumulation of slowly degradable roots and rhizomes and allochtonous organic matter trapped by the seagrasses (Kennedy et al. 2010, Duarte et al. 2013).

518 *Depth gradients in metabolic rates*

519 Shallow areas dominated by benthic vegetation maintaining large standing carbon stocks tend to
520 have a higher NEP compared to deeper waters dominated by phytoplankton of lower standing
521 stocks (e.g., Nixon et al. 2001, Caffrey et al. 1998, Caffrey 2004). In agreement with this, we
522 observed a clear trend with decreasing GPP, R and NEP from the shallow sites toward the central
523 deeper habitat. Differences in metabolic rates among the three investigated areas were much more
524 pronounced when comparing volumetric rates (rather than areal rates), which for GPP were 2.5
525 fold lower at the muddy deeper site compared to the two shallow sites, thus reflecting that the same
526 area-specific production was concentrated in a smaller volume at the shallow sites with benthic
527 dominance. Similar conclusions were made by Odum in 1967 who found a clear declining trend
528 in oxygen concentration with increasing depth, reflecting higher metabolic rates (on a volumetric
529 basis) in shallower waters, where temperatures are higher and plant pigments are more
530 concentrated (Kemp & Testa 2011). It is a general feature that the compression of the photic zone
531 from several meters in phytoplankton communities to centimeters-meters in macrophyte stands to
532 mm scale in dense microalgal mats is paralleled by a marked increase in volumetric photosynthesis
533 while areal photosynthesis remains remarkably constant (Krause-Jensen & Sand-Jensen 1998).
534 The observed shift from positive towards negative NEP with increasing depth and distance from
535 the shore also supports the concept that non-advective physical processes drive a net transport of
536 organic matter from shallow to deeper areas (Kemp et al. 1997, Van de Bogert et al. 2007).
537 While gradients in metabolic rates were observed with increasing depth and distance from the
538 dense eelgrass meadows, habitat differences were less obvious in the data set covering a temporal
539 range from April to December. As an example, the shallow eelgrass and bare (~unvegetated) areas
540 had almost identical magnitude and seasonality in GPP, R and NEP, despite the much lower

benthic rates measured here. The discrepancy between total rates and the sum of benthic and pelagic rates in the bare habitat, suggests that open-water rates measured in this habitat were strongly affected by excess oxygen produced in the nearby dense eelgrass meadow. Although the muddy central site had comparable higher pelagic contribution, there was also a discrepancy between total rates and incubations here, suggesting that the oxygen spillover effect from dense eelgrass beds can be an important feature affecting oxygen dynamics in an area that far exceeds the local meadow.

Importance of environmental conditions for temporal variability in metabolic rates

Despite the observed differences in day-to-day variability of metabolic rates among the shallow and deeper areas, they displayed similar temporal variation and magnitude in open-water metabolic rates, having quite similar responses to abiotic conditions in terms of increasing net autotrophy during calm, warm and sunny periods. Similar temporal variation and relationships with meteorological drivers characterize productive temperate lake ecosystems (Laas et al. 2012, Staehr et al. 2007, 2010). Other studies of estuarine ecosystem metabolism have, however, reported weak relationships between NEP and prevailing temperature (negative), light (positive) and wind (negative) conditions (Caffrey 2004, Hoellein et al. 2013, Murrell et al. 2018). Studies of GPP, R and NEP using the eddy correlation technique, have reported substantial day-to-day variability strongly coupled to variations in light and hydrodynamic conditions (Hume et al. 2011, Lee et al. 2017). Based on the eddy correlation technique, that takes into account the lateral exchange of oxygen, significant differences in metabolic rates have also been identified between a seagrass site and a nearby unvegetated site (Hume et al. 2011). This suggests that the parameterization of diel oxygen changes applied in our study is not sufficiently sensitive to separate such lateral exchanges

564 between neighboring sites. Interestingly, a recent study has shown how the difference between
565 open water and bottle incubations of DO can be used as a proxy of benthic contribution, which in
566 shallow seagrass-dominated environments can be important, indicating that the open-water
567 method can capture both water column and benthic processes (Murrell et al. 2018).
568
569 Inability of the open-water oxygen technique to account for lateral oxygen exchanges in
570 calculations of metabolic rates likely explains the apparently strong effects of wind observed at
571 the shallow sites (Fig. 8D and 9). The larger diel oxygen excursions at the shallow (2 to 3 m) sites
572 were almost instantly reduced to levels and variations corresponding to those of at the deeper (4
573 to 5 m) sites when wind conditions (wind speed and direction) forced a significant (~20 to 30 cm)
574 shift in water levels. Larger uncertainties in calculated metabolic rates are therefore likely to occur
575 on cold, windy and cloudy days where diel changes in oxygen are mostly governed by physical
576 forcing rather than by biological activity, which our model is insufficient to capture. We, therefore
577 excluded those extreme days with poor model performance ($r^2 < 0.2$), which typically represented
578 situations when water levels increased and surface irradiance was low, observed as drops in
579 calculated GPP (and NEP; not shown). Still after excluding days with poor model fit, there was a
580 strong negative effect of wind speed on apparent GPP and NEP (Fig. 8). The choice of models to
581 correct for the effect of wind on the exchange of oxygen across the air-water interface can affect
582 calculated metabolic rates (Marino & Howarth 1993). Sensitivity studies, however, show that this
583 issue is a greater problem in oligotrophic systems, with small diel variations in oxygen
584 concentrations around saturation (Staehr et al. 2010),which was seldom the case in the shallow
585 eelgrass-dominated habitat. Finally, as air-sea exchange is more effective in the surface layers
586 (Wannikhof et al. 2009), wind-induced turbulence has a proportionately larger effect on the

587 metabolic rate calculations in shallow than in deep water (Caffrey 2004). Other studies have
588 explained the negative effect of wind on metabolic rates as a result of lower light availability,
589 either due to deeper mixing of phytoplankton (e.g. Staehr & Sand-Jensen 2007, Hu et al. 2015),
590 elevated levels of suspended particles (e.g. Arfi et al. 1993) or co-variation with wind and reduced
591 surface light from cloud cover (Fisher et al. 2003). Elevated respiration caused by wind-induced
592 resuspension of labile organic matter from the sediment surface into the water column (Demers et
593 al. 1987) may also reduce NEP (Dokulil 1994). Nevertheless, our results indicate that a significant
594 dilution or spillover effect occurs between adjacent habitats. Except for periods with very limited
595 water movement, it therefore seems appropriate to think of the measured open-water metabolic
596 rates as apparent, rather than absolute rates of local production and respiration. While this is a
597 weakness of the open-water oxygen method for determining site-specific metabolic rates, it
598 supports the use of this technique when aiming to evaluate system-integrated rates across larger
599 heterogeneous areas as compared to compartment specific rates where incubation techniques are
600 preferable (Kemp and Testa 2011, Staehr et al. 2012b).
601
602 *Conclusions*
603 This study confirms the important functional and biogeochemical role of eelgrass meadows in the
604 coastal systems. Besides their local importance as biologically diverse and productive hot spots, a
605 considerable spillover of oxygen from seagrass meadows to neighboring areas imply an ecological
606 and biogeochemical impact extending beyond their physical coverage. Furthermore, this spillover
607 effect is not limited to oxygen, but concerns other biogeochemically relevant substances as well,
608 such as organic carbon and nutrients. Our study suggests that the extent of this footprint is strongly
609 affected by prevailing wind forcing (Fig. 10), but surface irradiance, local physical characteristics

610 (depth, fetch), sediment characteristics (sediment grain size, organic matter content), density of
611 aquatic vegetation and water quality (Secchi depth and Chl *a* concentration) also modulate this
612 effect. While other methods, such as the eddy correlation technique, are superior in accounting for
613 lateral exchanges, our study shows that open-water oxygen measurements in combination with
614 chamber incubations are well suited to assess the importance of different habitats and
615 environmental conditions for spatio-temporal variability in estuarine metabolism.
616

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Tables

Table 1. Overview of sampling program and subsequent modeling. Study sites are shown in Figure 1.

Study component	Study sites	Study period
Water quality sampling	Mud	May-Dec 2015
Metabolic rates - seasonal study		
- *Diel O_2 measurements (open)*	Mud, Eelgrass, Bare	Apr-Dec 2015
- *Benthic incubations*	Eelgrass, Bare	3 occasions: Apr, Jun, Aug 2015
- *Pelagic incubations*	Mud	14 occasions: Apr-Dec 2015
Metabolic rates - short term study		
- *Diel O_2 measurements (open)*	Depth gradient across habitat types	26. Apr-20. May 2016
Modeling of metabolic rates	Mud, Eelgrass, Bare	Jan-Dec

Table 2. GLMM model results for effects of habitats and seasons on differences in total (open water), pelagic and benthic (chamber) rates of gross primary production (GPP), respiration (R) and net ecosystem production (NEP). Significant levels ($p<0.05$) effects are highlighted in bold. Degrees of freedom (DF) for the numerator (Num) and the denumerator (Den) along with the F-values are shown.

Type	Variable	Effect	Num DF	Den DF	F-value	p
Total	GPP	**habitat**	**2**	**387**	**7.4**	**<0.001**
		month	**7**	**219**	**25.6**	**<0.001**
		habitat*month	14	387	1.1	0.352
	R	**habitat**	**2**	**381**	**4.5**	**0.011**
		month	**7**	**219**	**7.3**	**<0.001**
		habitat*month	14	381	1.2	0.244
	NEP	**habitat**	**2**	**381**	**4.4**	**0.013**
		month	**7**	**219**	**3.6**	**<0.001**
		habitat*month	14	381	1.4	0.155
Pelagic	GPP	month	6	7	2.0	0.187
	R	month	6	7	1.1	0.463
	NEP	month	6	7	1.3	0.369
Benthic	GPP	**month**	**2**	**14**	**30.1**	**<0.001**
		Habitat	**2**	**14**	**47.6**	**<0.001**
		month*Habitat	**3**	**14**	**13.7**	**<0.001**
	R	**month**	**2**	**14**	**12.8**	**0.001**
		Habitat	**2**	**14**	**15.3**	**<0.001**
		month*Habitat	**3**	**14**	**2.9**	**0.072**
	NEP	**month**	**2**	**14**	**13.2**	**0.001**
		Habitat	**2**	**14**	**4.0**	**0.043**
		month*Habitat	**3**	**14**	**4.2**	**0.026**

Table 3. Correlation table between daily values of water temperature (Tw), surface irradiance (PAR), wind speed at 10 m (Wind), gross primary production (GPP), ecosystem respiration (R) and net ecosystem production (NEP) at the shallow eelgrass site (Eelgrass) and non-eelgrass site (Bare) and the central deeper St60 (Muddy) habitat. The Spearman rank correlation coefficient (r_s) is shown with the significance levels (p).

Habitat	Parameter	GPP		R		NEP	
		r_s	p	r_s	p	r_s	p
Eelgrass	Tw	0.61	<0.0001	0.28	<0.0001	0.32	<0.0001
	PAR	0.53	<0.0001	0.15	0.0235	0.34	<0.0001
	Wind	-0.08	0.2491	0.15	0.0224	-0.18	0.0067
Bare	Tw	0.65	<0.0001	0.26	<0.0001	0.30	<0.0001
	PAR	0.52	<0.0001	0.09	0.1775	0.36	<0.0001
	Wind	-0.10	0.1362	0.29	<0.0001	-0.36	<0.0001
Mud	Tw	0.46	<0.0001	0.39	<0.0001	0.17	0.0187
	PAR	0.54	<0.0001	0.20	0.006	0.48	<0.0001
	Wind	-0.08	0.3125	0.25	0.0006	-0.36	<0.0001

838 Table 4. *In-situ* determined benthic rates (g O_2 m^{-2} d^{-1}) of gross primary production (GPP),
839 respiration (R) and net production (NEP) for three different habitats (Eelgrass, Bare sediment
840 and Muddy sediment) in Roskilde Fjord. Values are means of triplicates ± SD.

Habitat	Process	April	June	August
Eelgrass	GPP	0.4 ± 0.5	8.8 ± 2.6	3.5 ± 1.8
	NEP	0.2 ± 0.4	2.1 ± 1.5	-1.6 ± 4.2
	R	-0.2 ± 0.1	-6.7 ± 2.2	-5.1 ± 5.5
Bare	GPP	0.3 ± 0.9	1.7 ± 0.8	1.7 ± 3.7
	NEP	-0.2 ± 0.4	-0.4 ± 0.7	-2.4 ± 2.6
	R	-0.5 ± 0.6	-2.1 ± 1.4	-4.0 ± 5.0
Muddy	GPP	Nd	1.0 ± 2.4	0.3 ± 0.7
	NEP	Nd	-1.0 ± 1.6	-1.4 ± 1.4
	R	Nd	-2.0 ± 1.4	-1.8 ± 2.1

841

Figure legends

Figure 1. Location of the study sites in Roskilde Fjord, Denmark. Investigations were performed in two areas of the inner fjord with St60 representing the deeper (5 m) muddy (Mud) area, and the shallower (2-3 m) experimental area (Exp) representing a vegetated (Eelgrass) and a non-vegetated (Bare) site. The color scale in the central figure is a model estimate of the cover (0 to 100%) of eelgrass plants (Mohn et al. 2015).

Figure 2. Techniques applied to measure metabolic rates in different habitats: A) *in-situ* chamber for benthic oxygen fluxes, B) bottle incubations for pelagic primary production and respiration rates, C) buoy with oxygen sensor at the open-water muddy site (St60).

Figure 3. Seasonal changes in A) daily water temperature at 1 and 4 m depth and surface irradiance (PAR), B) daily wind speed 10 m above sea level, C) Secchi depth and Chl *a* (biweekly) and D) dissolved inorganic phosphorus (DIP) and nitrogen (DIN) (biweekly).

Figure 4. Daily estimates of gross primary production (GPP), ecosystem respiration (R) and net ecosystem production (NEP) determined from continuous oxygen measurements in two shallow (2-3 m) sites dominated by A) eelgrass and B) bare sediments, and C) in the deeper (5m) muddy site in Roskilde Fjord, Denmark (April-December 2015). R is presented as a negative number to facilitate plotting on the same graph as GPP and NEP. Negative NEP values are days when R outpaces GPP. Solid lines are 14-d moving averages. Mean GPP, R and NEP for the three sites over the study period are summarized in D) with 95% confidence intervals.

Figure 5. Comparison of GPP, R and NEP mean rates for the three studied habitats in Roskilde Fjord obtained from open-water oxygen measurements, representing total rates, and bottle incubations representing pelagic rates and benthic chamber rates. Means represent April-

November for total rates, April-September and December for pelagic rates, and April, June and August for benthic rates (cf. Fig. 5). Error bars show 95% confidence intervals of the means.

Figure 6. Monthly means of pelagic, benthic and open-water (total) rates of GPP, R and NEP in Roskilde Fjord estimated from the GLMM. Means represent an average of the three habitats and error bars show the 95% confidence interval of the mean values. Benthic rates marked with hatched bars were calculated as the difference between total and pelagic rates.

Figure 7. Differences in GPP, R and NEP rates across a shallow depth gradient in Roskilde Fjord. Rates are means over 24 days with 95% confidence interval.

Figure 8. Time series of A) wind speed and direction, B) water level and surface light, C) oxygen saturation level and D) GPP during a four week spring period in Roskilde Fjord. In C and D the shallow (1.8 to 2.1 m) and deeper part (4.1 to 5 m) of the estuary are contrasted, using data from the transect investigation. Shaded areas highlight periods with changes in water level related to changing wind speed and direction.

Figure 9. Relationship of wind speed with daily rates of A) GPP and B) NEP at shallow (1.8 to 2.1 m) and deeper sites (4.1 to 5 m).

Figure 10. Conceptual diagram for depth gradients in primary production and respiration from shallow nearshore to deeper central parts of Roskilde Fjord, under calm (left) and windy conditions (right). At shallower depths, light conditions favor benthic primary producers such as eelgrass in soft sandy habitats. These plants have higher areal GPP and a net positive oxygen balance. With increasing depth, planktonic and benthic microalgae capable of utilizing the high ambient nutrient levels and relatively high light levels dominate GPP and R. Muddy sediments at deeper depths prevent any significant establishment of macrophytes and contribute with high

886 oxygen demand, resulting in negative NEP. Increasing wind efficiently mixes water from deeper
887 areas onto shallow nearshore habitats, containing lower oxygen concentrations and higher levels
888 of suspended matter, thereby reducing water transparency and elevating pelagic oxygen
889 consumption.

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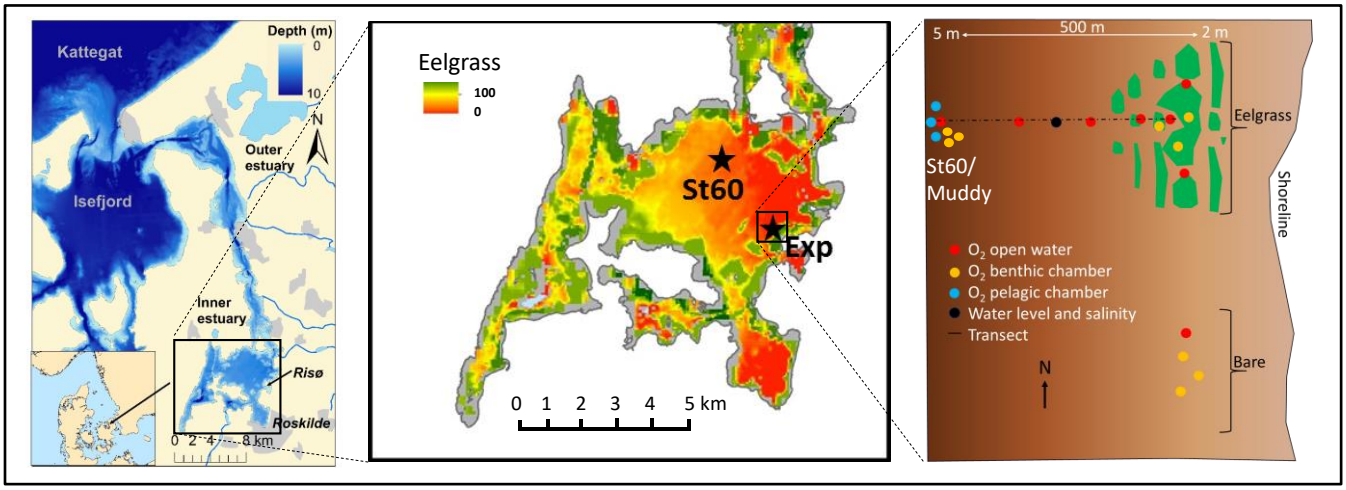
Figures

Figure 1.



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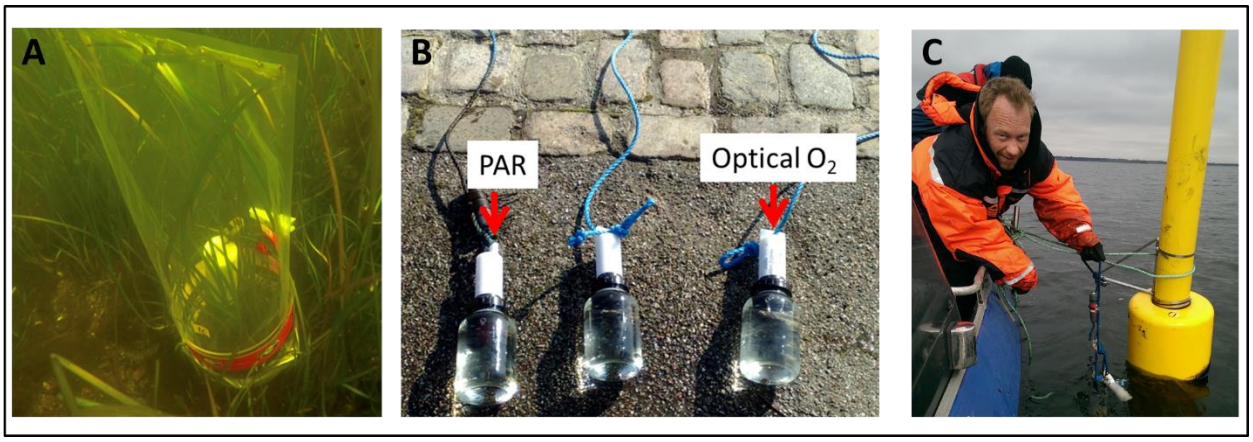

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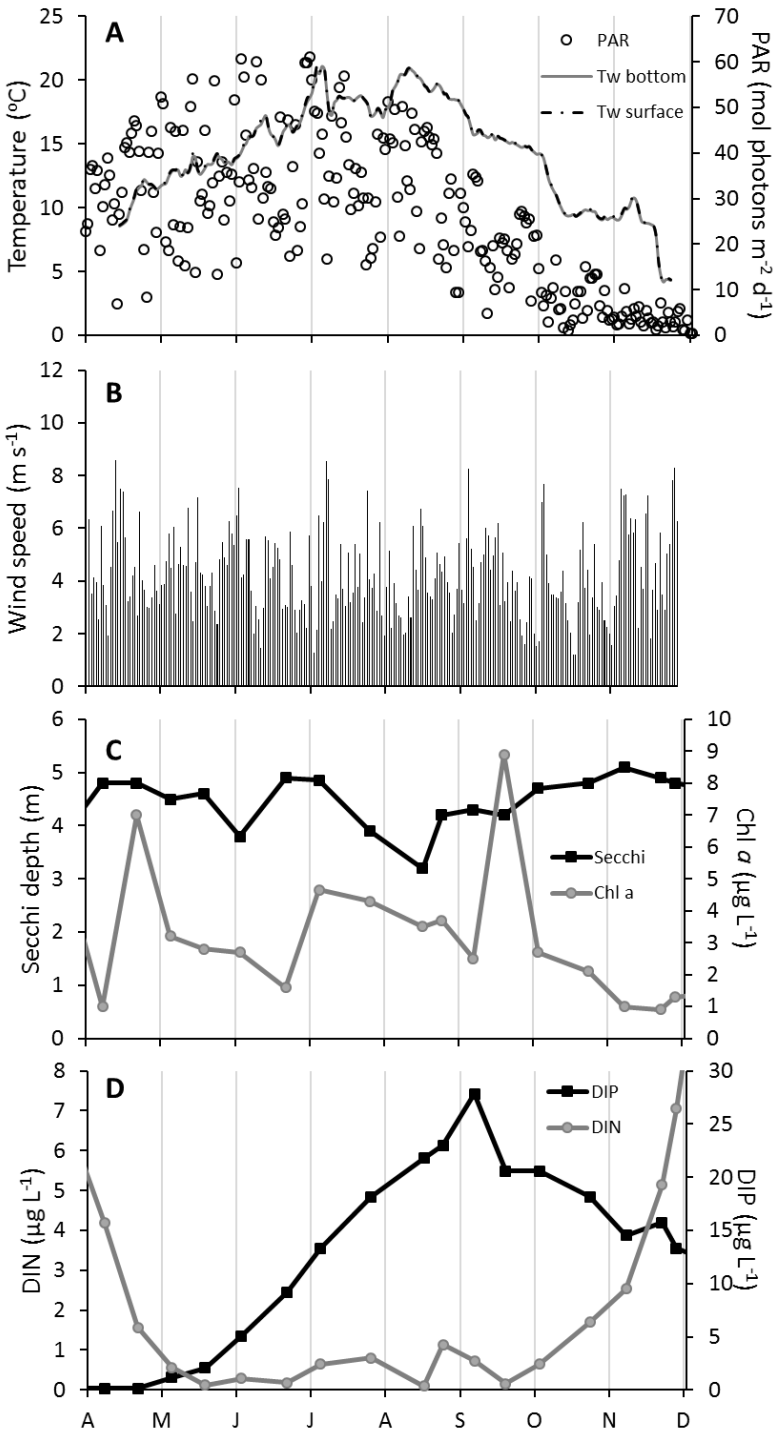

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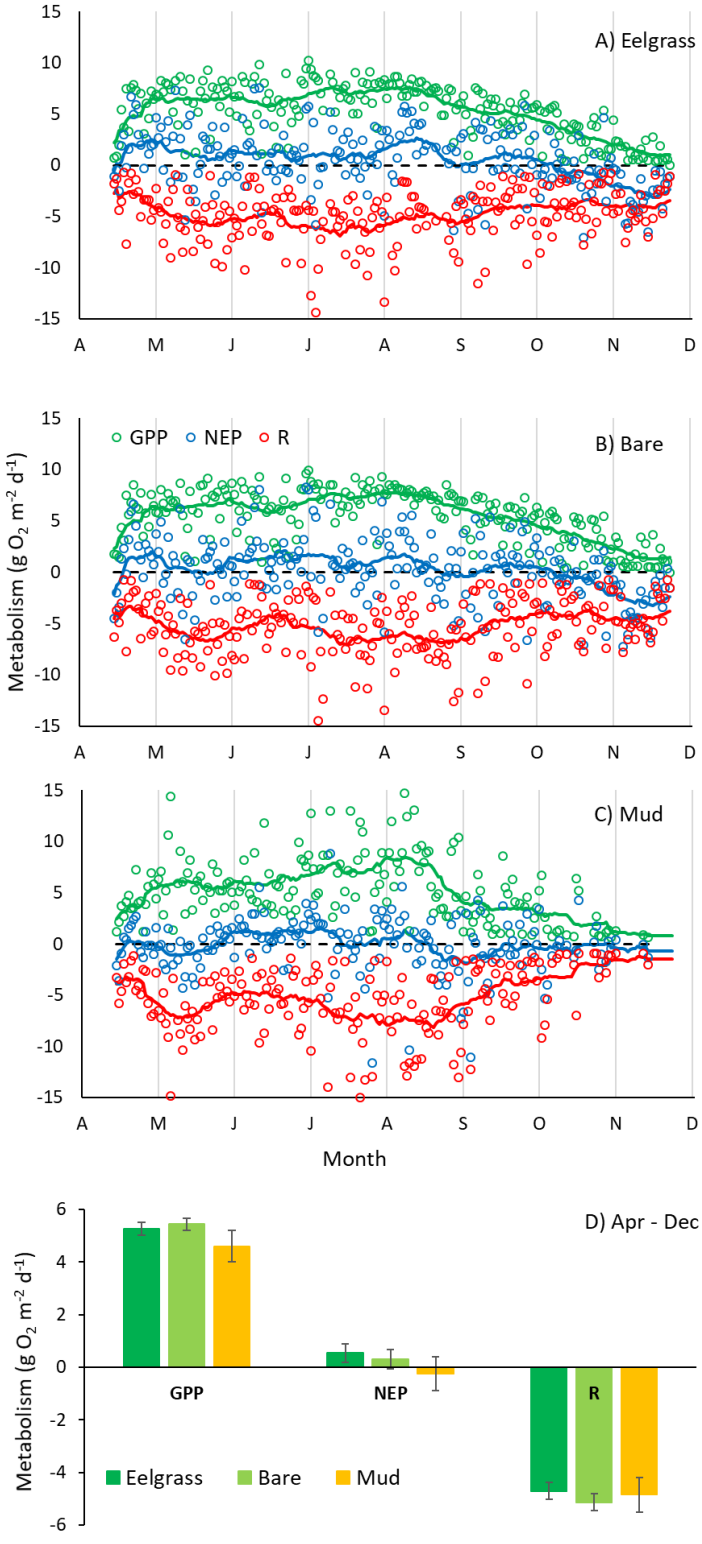

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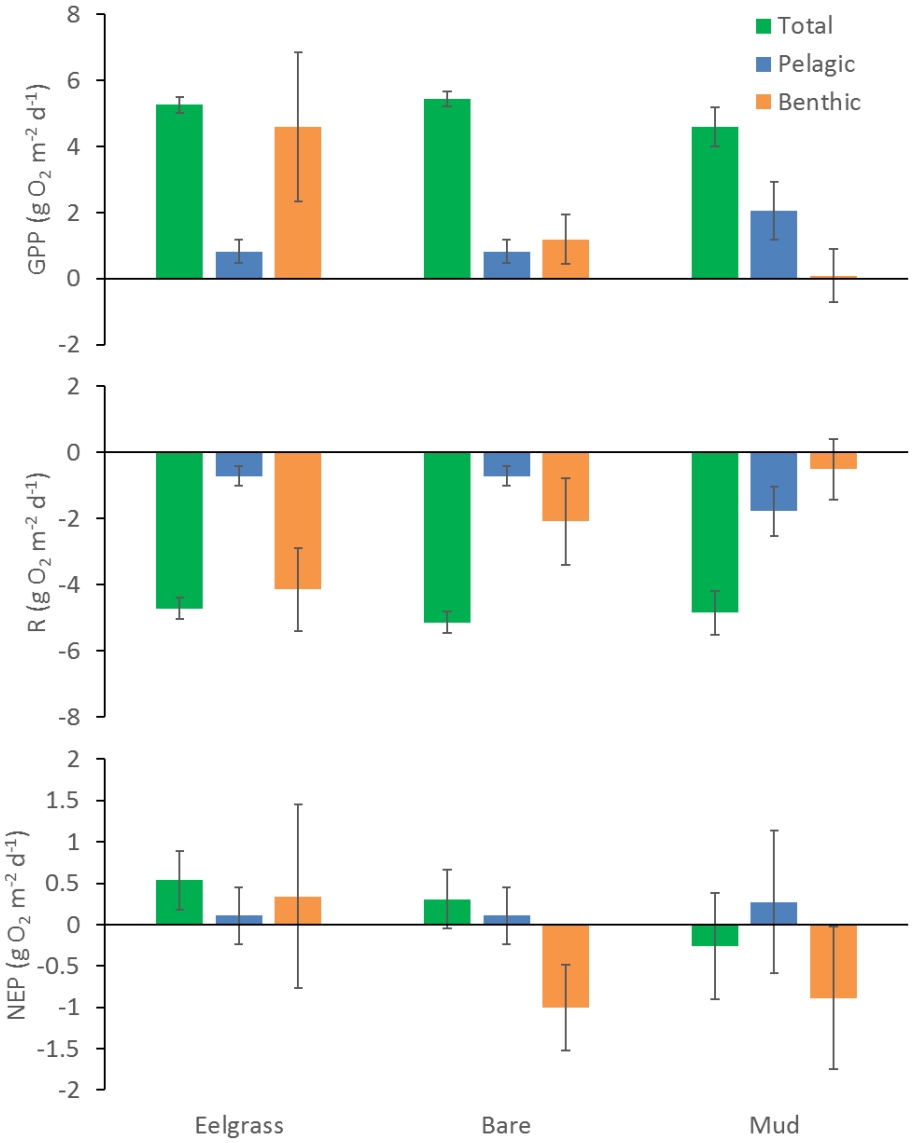

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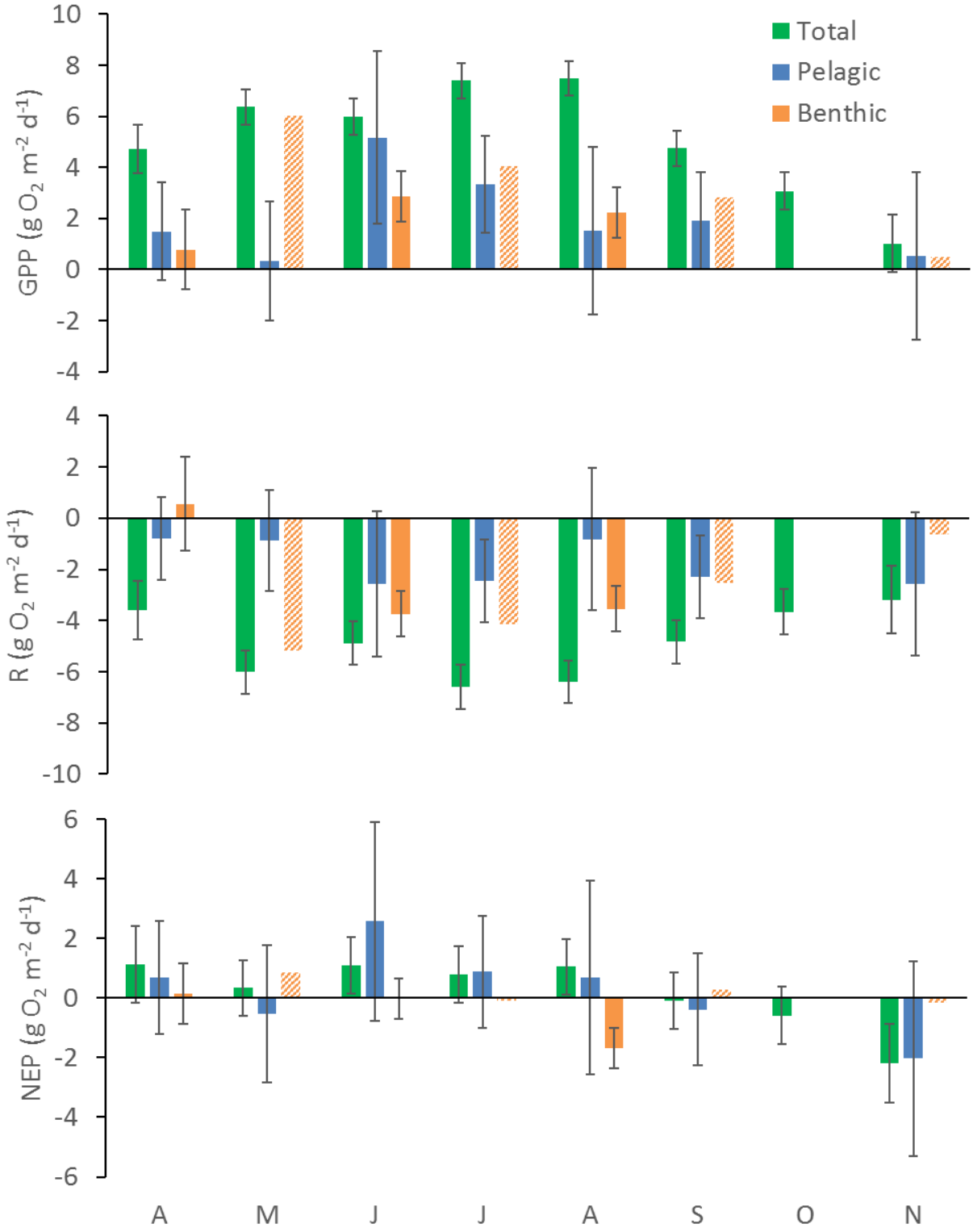
Figure 6.


Total
Pelagic
Benthic
GPP (g O2 m-2 d-1)
R (g O2 m-2 d-1)
NEP (g O2 m-2 d-1)
10
8
6
4
2
0
-2
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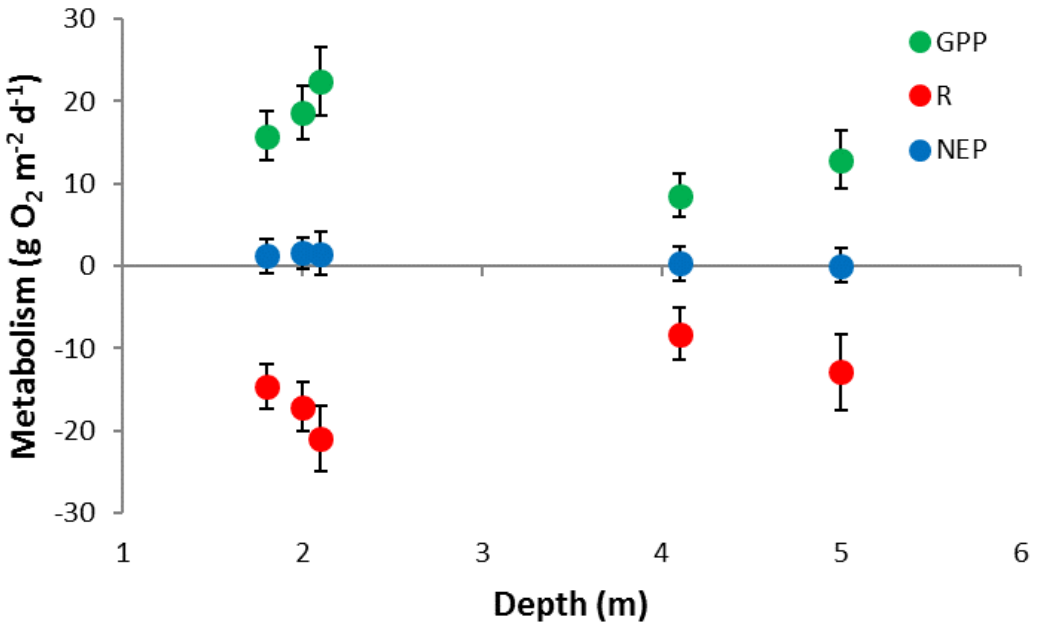
920 Figure 7.



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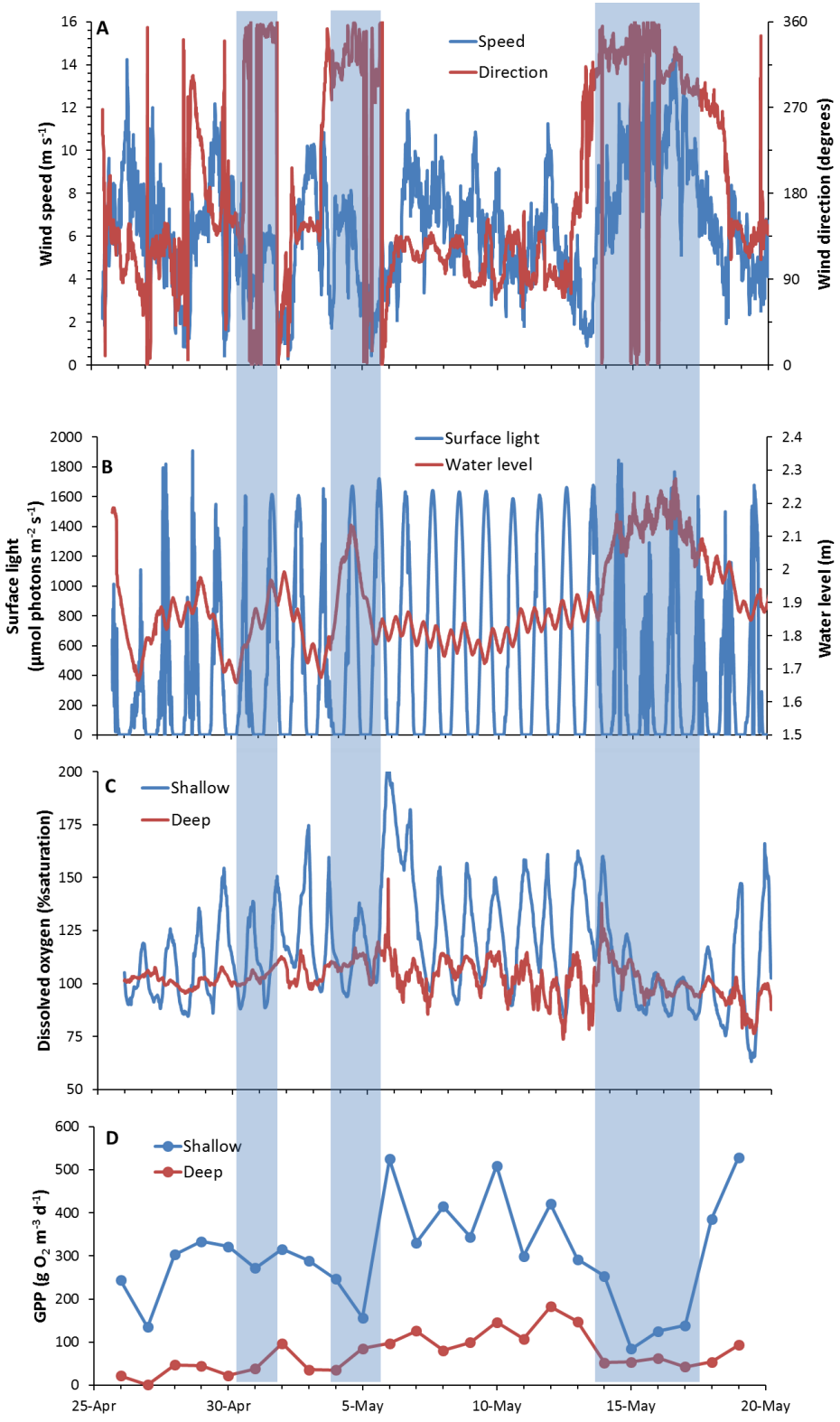

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925 Figure 9.

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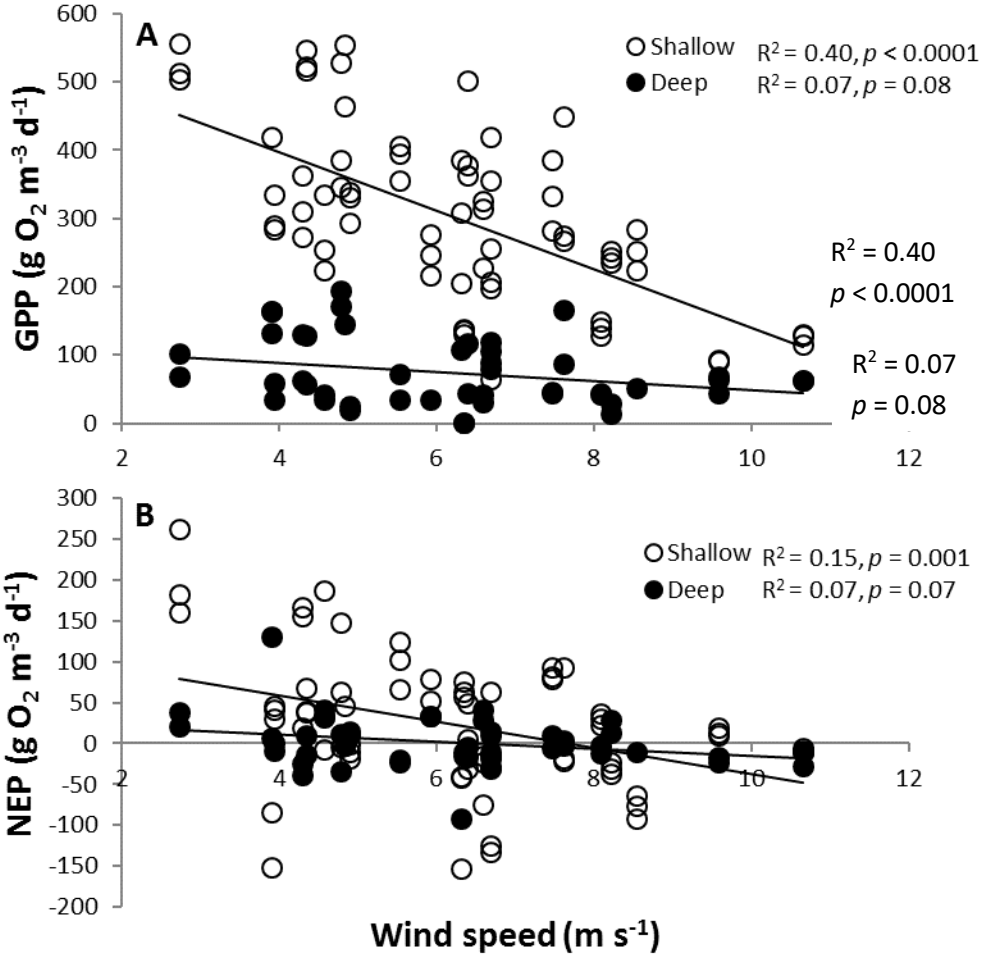

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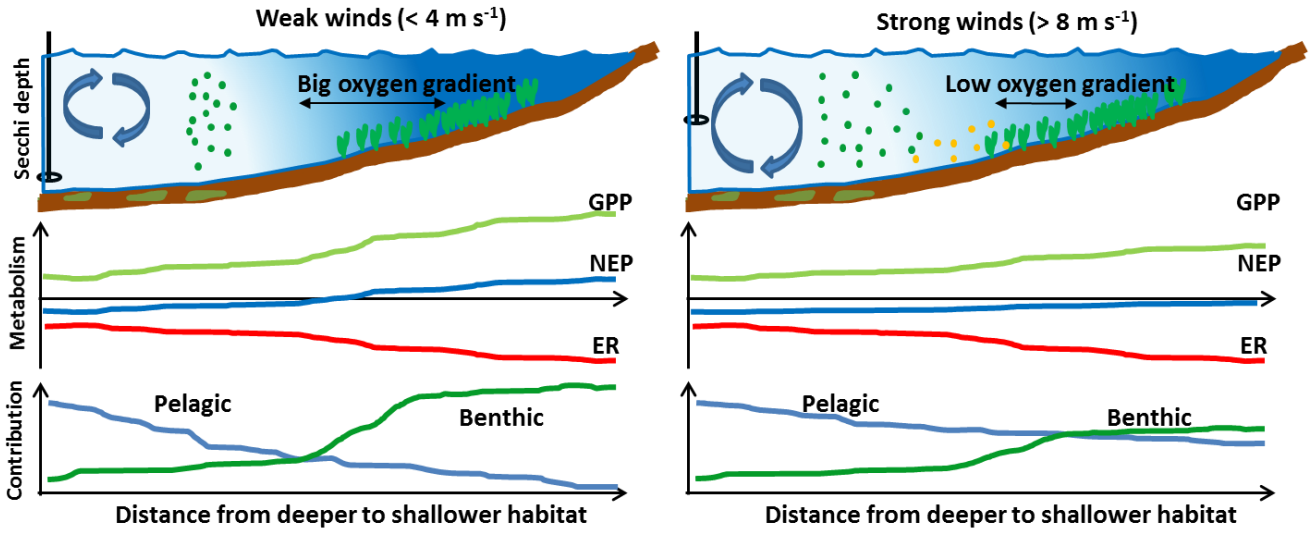

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